

SOME EXPERIMENTAL FISH HOSTS OF THE STRIGEID TREMATODE
BOLBOPHORUS CONFUSUS, AND EFFECTS OF
TEMPERATURE ON THE CERCARIA
AND METACERCARIA

by

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ABSTRACT

Experimental studies were conducted on the digenetic trematode, Bolbophorus confusus (Krause, 1914) Dubois, 1935, to test host specificity in the fish host and the effect of temperature on the cercaria and metacercaria.

Typical infections followed laboratory exposures of the following fish: Salmo gairdneri, Salmo trutta, Salvelinus fontinalis, Prosopium williamsoni, Pimephales promelas, Hybopsis gracilis, Rhinichthys cataractae, Catostomus commersoni, Pantosteus platyrhynchus, Lepomis macrochirus, and Gambusia affinis. Cercarial penetration occurred in Ictalurus punctatus and Cottus bairdi, but metacercarial development was atypical.

Maximum cercarial penetration of fish occurred between 66 and 85°F. Invasive ability was markedly reduced between 55 and 65°F and very few penetrations occurred below 54°F.

Metacercariae developed rapidly at 70°F. No metacercarial development was found in fish exposed to cercariae at 70°F and then held at 40-42°F. Some metacercariae were observed to develop normally when fish treated in the above manner were returned to 70°F, after being held at 40-42°F for one month.

Water temperature apparently plays a major role in the distribution of the metacercaria of B. confusus (common only in one lake) in southwestern Montana.

INTRODUCTION

The digenetic fluke Bolbophorus confusus (Krause, 1914) (Dubois, 1935), is established in Meadow Lake in southwestern Montana (Fox, 1962). Although this fluke is reported to be common in Europe, the metacercaria had not been previously reported in North America. It has not been found in other lakes in the Meadow Lake vicinity. In Europe, Dubois (1938) reported the metacercaria of this parasite in the following fish: rudd (Scardinius erythrophthalmus), id (Idus idus), bream (Abramis brama), flat bream (Abramis blicca), northern pike (Esox lucius), perch (Perca fluviatilis) and mullet (Mugil saliens). He reported the final hosts to be the pelicans Pelecanus onocrotalus and Pelecanus crispis in Europe, and the white pelican (Pelecanus erythrorhynchos) in North America.

In Meadow Lake, the metacercariae are found in rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), arctic grayling (Thymallus arcticus), longnose sucker (Catostomus catostomus) and white sucker (Catostomus commersoni).

Fox (1962) observed a marked difference in the number of B. confusus metacercariae found in brown and rainbow trout from Meadow Lake. All brown trout examined were infected, with an average of 194 cysts per fish. Cysts were found in 25 of 30 rainbow trout examined and averaged only 34 per fish. The difference in the degree of infection between the trout and the fact that B. confusus metacercariae are not reported in fish from nearby lakes, formed the basis for this study.

The objectives were: to study the host specificity of B. confusus metacercariae in fish, and to find an explanation for the prevalence of the parasite in Meadow Lake fish.

Although the snail host and cercaria of B. confusus were not known at the time the parasite was found in Meadow Lake, the complete life cycle has since been determined by Fox (1964). It is briefly as follows: The adult fluke is found in the anterior intestine of the white pelican. The eggs pass out with the feces into the water and hatch after about 15 days. The resulting ciliated miracidia penetrate the planorbid snail, Helisoma trivolvis. Sporocysts develop in the snail producing large numbers of cercariae. After leaving the snail, the cercariae penetrate fish and encyst in the musculature as metacercariae. When eaten by a pelican, the metacercariae develop to patent adults within a few days.

MATERIALS AND METHODS

Numerous attempts were made to collect Helisoma trivolvis snails from Meadow Lake in the spring and early summer of 1963, but none were found until August, when they were abundant. The reason for this apparent absence of H. trivolvis snails is not known. Snails of the genera Physa and Gyrulus were found commonly throughout the summer.

Infected H. trivolvis snails were found but the prevalence was extremely low. To obtain the desired numbers of cercariae, it was necessary to infect snails in the laboratory. B. confusus eggs were obtained from pelicans collected at Meadow Lake. When the eggs hatched, 1 to 4 miracidia were placed in a stender dish with an uninfected snail.

Snails producing cercariae were kept in finger bowls containing tap water that had been aged for several days to remove chlorine. Snails were satisfactorily maintained on a diet of boiled lettuce.

Freshly shed cercariae for experimentation were obtained by placing snails in finger bowls containing clean water for a period of up to 24 hours before experiments began. When at least several hundred were shed, the finger bowls were emptied into a 1.5 gallon aquarium. Additional water was added to the aquarium until the total volume equaled 2000 ml. Cercariae were seen to be randomly dispersed throughout the water when a light was placed behind the aquarium. The approximate number of cercariae in the aquarium was determined by taking four 10 ml.

samples, counting the number in each sample under a dissecting microscope and projecting to the total volume.

Experimental fish were obtained from several sources. The following fish were taken from lakes and streams in southwestern Montana: rainbow trout, brown trout, brook trout (Salvelinus fontinalis), mountain whitefish (Prosopium williamsoni), long-nose dace (Rhinichthys cataractae), flathead chubs (Hybopsis gracilis), mottled sculpins (Cottus bairdi), mountain suckers (Pantosteus platyrhynchus) and white suckers. Mosquitofish (Gambusia affinis) were collected from a small warm spring in western Montana. Fathead minnows (Pimephales promelas), bluegills (Lepomis macrochirus), channel catfish (Ictalurus punctatus) and some rainbow trout were obtained from hatcheries. A number of fish from each source was examined for parasites and in no case were any found. All fish, except mosquitofish, were held in hatchery troughs containing cold (40-54°F) running water until used in experiments. Mosquitofish were kept in aquaria at higher temperatures.

Fish were exposed to the parasite in the 1.5 gallon aquarium containing cercariae. This was done to prevent mechanical damage to the cercariae and also to keep them concentrated in a small volume. Hereafter this 1.5 gallon aquarium is referred to as the exposure chamber. Prior to exposure the fish were placed in an aquarium where the water temperature was gradually adjusted to that of the exposure chamber. When this temperature

was reached, the fish were placed in the exposure chamber. The time of exposure ranged from 15 minutes to 16 hours, but was 30 minutes in most experiments. After exposure, the fish were held in aquaria at the temperature desired.

Prior to examination, total lengths of all exposed fish were obtained to the nearest quarter inch. Exposed fish were examined with the aid of a dissecting microscope. The smaller specimens (1 to 3 inches) were examined whole after carefully teasing apart the flesh. The larger specimens (3 to 7 inches) were cut into two parts along the median sagittal line and both parts examined. All metacercariae were counted and the development noted.

LABORATORY OBSERVATIONS ON THE CERCARIA AND METACERCARIA

The furcocercous distome cercaria of B. confusus hangs vertically in the water, with the body downward. When not disturbed, it moves only occasionally and then with a vibratory motion that carries it upward. The attitude assumed in the water and the type of motion exhibited is similar to that observed for the cercaria of Hysteromorpha triloba (Hugghins, 1954), Crassiphiala bulboglossa (Hoffman, 1956) and Diplostomum baeri eucaliae (Hoffman and Hundley, 1957). The body is approximately 420 μ in length and 30 μ in width. The furcae are approximately 240 μ in length.

Fox (1962) found the lemon-shaped host cysts to be 850-1500 μ long and 55-1000 μ wide. The average length of metacercariae was 2341 μ . The sizes of host cysts and length of mature metacercariae that I observed were comparable.

EXPERIMENTAL RESULTS

During the winter of 1963-64, 13 species of fish were exposed to cercariae and the effect of temperature upon cercarial penetration and metacercarial development was studied. It was determined that in exposed fish held at 70°F metacercariae are readily found, but are small and unencysted after 12-15 days. The parasite cyst is present at about 18-20 days and mature metacercariae enclosed in host cysts develop in 25-30 days. This was considered to be normal laboratory development time when comparing development in infected fish.

Laboratory Infection of Various Species of Fish

The criterion used to determine possible intermediate hosts was normal development of the metacercariae in the fish. Unless otherwise stated, fish were exposed and held at 70-72°F and exposed for 30 minutes. To allow for metacercarial development, only fish that lived 10 days or more after exposure were used.

Salmonidae. Three experiments, each with 2 rainbow and 2 brown trout, were carried out. Rainbow trout ranged in length from 3 to 6 inches and the brown trout from 4 to 5.5 inches. The average number of cercariae per exposure was 900. All of the trout exhibited irritation during exposure by erratic swimming and by attempting to jump out of the exposure chamber.

Normal metacercariae were recovered from all of the trout with the exception of one brown trout that died the day after exposure. The average number of metacercariae recovered from

brown trout was 40, and from rainbow trout, 53.3.

In addition, 7 experiments were done involving a total of 15 rainbow trout ranging from 3.5 to 5 inches in length. An average of 1900 cercariae was used. Reaction to cercariae was identical to that described above. An average of 49.4 normal metacercariae were recovered from each fish.

Three brook trout, 4 to 4.5 inches in length, were exposed to approximately 800 cercariae in one experiment. Irritation due to cercariae was indicated by erratic swimming, but no jumping was observed. Normal metacercariae developed in all brook trout and averaged 26.6 per fish.

A 7-inch mountain whitefish was exposed to approximately 1800 cercariae. No apparent irritation due to the cercariae was observed, but 125 normal metacercariae were recovered.

Cyprinidae. Five experiments were done using a total of 20 fathead minnows that ranged from 1 to 2.25 inches in length. An average of 2000 cercariae was used per exposure. The minnows exhibited irritation during exposure by short jerking movements and by turning on their sides to rub the bottom of the chamber. Normal metacercariae developed and averaged 23.2 per fish.

Three longnose dace, 3 to 4 inches long, were exposed to a concentration of 3800 cercariae for 15 minutes. They often turned on their sides and rubbed against the bottom and against each other during exposure. All became infected with an average of 54 metacercariae per fish. Metacercariae were small and

unencysted 19 days after exposure, but this was probably because the fish were held at 64°F after exposure.

Two flathead chubs, 4.5 and 5 inches long, were exposed to approximately 400 cercariae in one experiment. Normal metacercariae were recovered from each fish (38 and 43 respectively).

Catostomidae. In three experiments, 8 white suckers (1.5 to 2.25 inches long) were exposed to an average of 2600 cercariae. The suckers exhibited extreme irritation throughout the exposure period by rubbing on the bottom and against each other. Development of the metacercariae was normal in all fish, and the average recovered was 27.7 per fish.

Two mountain suckers, 5.5 and 6 inches in length, were exposed to approximately 400 cercariae in one experiment. They did not appear at all irritated by cercariae, but normal metacercariae were found in both (27 and 17 respectively).

Centrarchidae. Nine bluegills, 1 to 1.25 inches long, were exposed to approximately 1200 cercariae at 64°F. Irritation due to cercariae was indicated by erratic swimming, but no rubbing was observed. The fish were held at 64°F after exposure. Seven became infected, but an average of only 6 small metacercariae was recovered per fish. As with the longnose dace, the lower temperature may have been a factor in retarding metacercarial development.

In a later experiment, a 1.25 inch bluegill was exposed for one hour to approximately 1300 cercariae at 74°F and held at 70°F.

Metacercarial development was normal and 31 were found.

Poeciliidae. A total of 10 mosquitofish, 1 to 1.5 inches long, were exposed to an average of 600 cercariae in 3 experiments. They exhibited irritation by frequent rubbing against the bottom and against each other. Metacercariae recovered were normal and averaged 5 per fish. Although 4 fish were exposed for one hour and 6 for 30 minutes, the average number of metacercariae recovered was similar for both lots.

Ictaluridae. Six channel catfish, ranging in length from 2 to 2.5 inches, were exposed to approximately 1200 cercariae in one experiment. The exposure and holding temperature was 64°F. No metacercariae were found 14 to 16 days after exposure. The bluegills (previously mentioned) exposed in the same experiment and held at the same temperature became infected. This indication that channel catfish are refractory to infection was investigated in an experiment conducted under optimum conditions for the parasite. One 2.25 inch catfish was placed in a small jar that contained approximately 1000 cercariae. Sixteen hours were allowed for exposure at a temperature of 72°F and no irritation was apparent. The fish was examined after being held for 25 days at 70°F and 55 metacercariae were found. Only one was encysted and normal in size and development. The remainder were unencysted, small and poorly developed. Channel catfish are apparently abnormal hosts, although cercarial penetration will occur under ideal conditions.

Cottidae. Four mottled sculpins, 2.5 to 3.5 inches long, were exposed to approximately 800 cercariae in one experiment. Twenty days after exposure, 2 sculpins died and were found to contain 12 and 36 poorly developed, unencysted metacercariae. Thirty days after exposure, the 2 remaining sculpins appeared healthy and contained 1 and 3 metacercariae. The metacercariae were encysted, but were abnormal in shape and unusually small.

Possible reasons for the marked difference in the number of metacercariae found in the sculpins that died after 20 days and those examined after 30 days are: The parasites were not able to become established in the sculpins and degenerated between 20 and 30 days, or fewer cercariae penetrated the sculpins that lived for 30 days. In either case, it appears that the mottled sculpin is an abnormal host.

Based on the criterion used to indicate suitable hosts, only channel catfish and mottled sculpins, of the species tested, are doubtful intermediate hosts of B. confusus (Table I).

Temperature and Cercarial Penetration

In initial experiments, difficulty was encountered in infecting fish with the parasite. Two factors believed to be possible causes of this were temperature and exposure time. A series of experiments was conducted to test the factors and results are presented in Table II. Only those species of fish in which metacercarial development was known to be normal were

TABLE I. Experimental hosts of Bolbophorus confusus

Species	Number of fish exposed	Number of fish infected	Metacercarial development
Rainbow trout	21	21	Normal
Brown trout	5	5	Normal
Brook trout	3	3	Normal
Mountain whitefish	1	1	Normal
Fathead minnow	20	20	Normal
Longnose dace	3	3	Normal
Flathead chub	2	2	Normal
White sucker	8	8	Normal
Mountain sucker	2	2	Normal
Bluegill	10	8	Normal
Mosquitofish	10	10	Normal
Channel catfish	7	1	Abnormal
Mottled sculpin	4	4	Abnormal

TABLE II. The effect of temperature on cercarial penetration

	Temperature Ranges		
	48-54°F	55-65°F	66-85°F
No. of experiments	4	6	21
No. of fish	26	33	71
Avg. length (inches)	1.9	1.7	2.8
Avg. exposure time (minutes)	83	36	29
No. of cercariae used			
Range	200-6000	500-3000	50-4000
Average	2400	1500	1800
No. of metacercariae recovered			
Range	0-6	1-28	1-125
Average	0.8	9.7	35.9

used. The numbers of metacercariae recovered were used to indicate the numbers of cercariae that penetrated the fish.

The time of exposure varied from 15 to 180 minutes, generally being longer at the lower temperatures. The longer time at the lower temperatures was allowed to give the cercariae greater opportunity to penetrate fish at these temperatures.

Three arbitrary temperature ranges were used for exposure. These ranges were: 48-54°F, 55-65°F, and 66-85°F. All fish were held at 70-72°F after exposure. The data obtained in each range were averaged for comparison. All fish in the 48-54°F and 55-65°F ranges were examined 2 weeks or more after exposure to insure recovery of all metacercariae. Some fish exposed at 66-85°F were examined 8 days after exposure, so all metacercariae may not have been recovered because of their small size. Therefore the average number of metacercariae for the highest temperature range is a minimum.

The data show a decline in the number of cercariae penetrating fish from the higher temperatures (avg. 35.9 metacercariae) to the lower temperatures (avg. 0.8 metacercariae).

Temperature and Metacercarial Development

A number of experiments were designed to investigate the effect of temperature on metacercarial development in suckers and minnows (Table III) and in rainbow trout (Table IV). All fish were exposed to varying numbers of cercariae at 70°F for 30

TABLE III. The effect of temperature on metacercarial development in white suckers and fathead minnows

No. of fish (total)	22 suckers and 22 minnows
Length range (inches)	1.5 to 2.25
Avg. no. of cercariae used	3000
Group I	
No. of fish	16
Mortality	0
Avg. no. of metacercariae per fish	0.1
Group II	
No. of fish	11
Mortality	0
Avg. no. of metacercariae per fish	4
Group III	
No. of fish	17
Mortality	100%
Avg. no. of metacercariae per fish	36.5

TABLE IV. The effect of temperature on metacercarial development in rainbow trout

No. of fish (total)	32
Length range (inches)	3.5 to 5.5
Avg. no. of cercariae used	2100
Groups I and II	
No. of fish	20
Mortality	75%*
Avg. no. of metacercariae per fish	0
Group III	
No. of fish	12
Mortality	100%
Avg. no. of metacercariae per fish	61.8

*Mortality not attributed to the parasite.

minutes. After exposure, the fish were divided into 3 groups. Group I fish were held at 40-54°F; group II fish were held at 40-42°F for about a month, and then returned to 70°F for several weeks; group III fish were held at 70°F. Holding time varied within the groups because of mortality.

Four experiments were conducted using white suckers and fathead minnows (Table III). No mortality occurred in group I fish. One metacercaria was recovered from each of 2 fish; the larvae were encysted, but abnormally small. No metacercariae were recovered from the remaining 14 fish in this group. Hemorrhages were not observed in any fish. Mortality did not occur in group II fish. Metacercariae were recovered from 10 of the 11 fish and the average per fish was 4. No hemorrhaging was observed. All fish in group III died in 6 to 16 days (avg. 11 days). Extensive hemorrhaging was observed throughout the musculature and normal metacercariae were recovered from all fish (avg. 36.5 per fish).

Since most group II fish and 2 group I fish developed infections, survival at low temperatures for at least 30 days is possible for some of the larvae. However, little development occurs at these temperatures. The lack of development may account for the failure to recover more metacercariae from group I fish. All group III fish died, but no mortality occurred in groups I and II. The rate of metacercarial development was apparently a factor in the mortality.

Six experiments were carried out using rainbow trout. Initially, groups I and II were separated, however, 50% mortality in both groups occurred within 21 days at low temperatures. The reason for the mortality was believed to be the stress resulting from changes in temperature. The fish remaining were combined (Table IV) and treated as group II fish to determine if metacercariae would develop. Five group II fish died 3 to 11 days after return to 70°F, the remainder lived until examined after 24 days at 70°F. No hemorrhaging was observed and no metacercariae were found in any of the fish that died at low temperatures or those that were raised to 70°F. All fish in group III died in 8 to 26 days (avg. 13 days). Hemorrhaging was very heavy in 7 fish that did not live over 12 days, but not in fish that lived longer. Normal metacercariae were recovered from all fish (avg. 61.8 per fish).

DISCUSSION AND CONCLUSIONS

Host Specificity

The wide variety of fish species infected in this study and the number of species known to serve as intermediate hosts in Europe indicate little host selectivity on the part of the cercariae. Some host specificity was indicated by abnormal metacercarial development in channel catfish and mottled sculpins.

The difference in the infection of brown and rainbow trout that Fox (1962) reported was not found in the laboratory. The rainbow trout he examined were believed to be mostly hatchery fish (9-10 inches when planted) and the average length was smaller than the brown trout. The larger size and longer potential exposure period may be the reason more metacercariae were found in brown trout.

Distribution of the Metacercariae

The factor that limits a parasite to a specific location is often the absence of one or more of the hosts from other apparently suitable areas. Many lakes in the Meadow Lake vicinity, however, do support the proper bird, snail and fish hosts. White pelicans frequent many lakes in southwestern Montana and H. trivolvis snails are widely distributed in this area. Although stages of the parasite life cycle probably are present in many of the lakes, the prevalence in fish (if present) is low.

The temperature studies show decreased activity of the cercariae (as evidenced by low infections) and slow development of the metacercariae at temperatures under 65°F. Temperatures below optimum for these larval stages may be the reason the metacercariae are common only in Meadow Lake.

Other investigators have found that water temperature has an effect on the development and activity of the larval stages of parasites. Vogt (1938) found that the procercooids of Triaenophorus nodulosus develop faster at 58°F than at 39°F. DeGiusti (1949) observed that larval development of Leptorhynchoides thecatus in the amphipod takes 2 months at 55 to 59°F and one month at 68 to 75°F. According to Bevelander (1934) the frequency of contraction of the caudal fork of Bucephalus elegans cercariae increases from 0 to 102 per minute when temperature increases from 32 to 82°F. Pigulevskii (1953) found that the miracidium of Phyllodistomum folium is very active at 63 to 68°F, but at 41 to 45°F its movements abruptly slow down. Summarizing the effect of temperature, Bauer (1962) stated that temperature is an exceptionally important factor in the life of freshwater fish parasites. It determines not only the range of the given species, but also its abundance within the range.

Complete temperature information is not available for lakes in this area, especially for shallow, protected bays where the snails are usually found. However, some general comparisons of a few lakes may be made.

Meadow Lake is an impoundment on the Madison River located about 5 miles north of Ennis, Montana. The lake lies at an elevation of 4840 feet and has a surface area of 1945 acres. The maximum depth is 38 feet, but is mostly less than 15 feet (Fox, 1962). Ice usually leaves the lake by the middle of April. The lake is often wind-swept and complete water mixing results in little variation of temperature from surface to bottom. According to Heaton (1962) the maximum temperature recorded in Meadow Lake during the summer of 1961 was 75°F. In the months of July and August, bottom temperatures did not drop below 65°F and were usually near 70°F. Summer temperatures in Meadow Lake are therefore optimum for cercarial penetration and metacercarial development for at least 2 months.

Hebgen Lake, an impoundment on the Madison River, lies about 50 miles upstream from Meadow Lake at an elevation of 6544 feet. Ice usually disappears in late April or early May (Graham, 1961). Before the 1959 earthquake, it had a surface area of 13,700 acres and a maximum depth of 61.5 feet (Heaton, 1961). The highest surface water temperature recorded during 1953-54 was 76°F (Graham, 1961) and during the summer of 1961 was 68°F (Heaton, 1962). For most of the summer, surface water temperatures were in the mid-60 degree range and lower (Heaton, 1962). The highest temperatures recorded in Hebgen Lake are similar to those recorded in Meadow Lake. However, the average temperatures in Hebgen Lake are lower and the period of high temperatures

is shorter. Although the temperature is not low enough to eliminate the parasite, it could retard development and activity of the parasite (miracidium, cercaria and metacercaria) so the cycle could not be completed during the warm water period.

Quake Lake, located less than 2 miles below Hebgen Lake on the Madison River, has a surface area of approximately 1000 acres and a maximum depth of 140 feet (Heaton, 1961). It is supplied by cold water from the lower depth of Hebgen Lake. Water temperatures are lower (maximum during summer of 1961 was 64°F) than those recorded in Hebgen Lake (Heaton, 1962), so the possibility of the parasite becoming established is low.

Yellowstone Lake has a surface area of 88,478 acres, a maximum depth of 320 feet (mean 139 feet) and lies at an elevation of 7731 feet (Benson, 1961). Ice does not leave the lake until late May or early June. The lake supports a large pelican rookery and birds from here probably visit the other lakes mentioned. Water temperatures were obtained over a 5 year period by Benson (1961). The maximum surface water temperature recorded was 64°F and minimum was 41.2°F. Summer temperatures were usually in the 50 degree range. These temperatures would retard development and activity of the various larval stages of the parasite so the possibility of establishment of the parasite is very low.

Mortality

Mortality of fish infected with the parasite occurred in

the laboratory, particularly in experiments using heavy concentrations of cercariae (1500 plus) at optimum temperatures. Hemorrhaging in fish that died was very extensive, apparently because of rapid metacercarial development. Little or no mortality attributed to the parasite occurred in fish from experiments using low concentrations of cercariae (less than 1500), or from experiments using below optimum temperatures. Severe hemorrhaging was not produced, apparently because the lower temperatures slow metacercarial development. Periodic summer fish mortalities have occurred in Meadow Lake, but the causes are unknown. If high water temperatures optimum for the parasite reached a point critical for the fish under parasite-free conditions, the presence of the parasite may have been a factor contributing to the fish mortality. Natural infections probably occur most often over an extended period of time and at temperatures not always optimum for the parasite. This could enable the fish to better withstand the stress of being parasitized with B. confusus metacercariae.

SUMMARY

1. B. confusus cercariae were obtained from H. trivolvis snails infected in the laboratory.
2. Metacercarial development was normal in the following fish after exposure to cercariae in the laboratory: rainbow trout, brown trout, brook trout, mountain whitefish, fathead minnow, flathead chub, longnose dace, white sucker, mountain sucker, bluegill, and mosquitofish.
3. Cercarial penetration of channel catfish and mottled sculpin occurred, but metacercarial development was not normal.
4. Maximum cercarial penetration of fish occurred between 66 and 85°F. Invasive ability was markedly reduced between 55 and 65°F and very few penetrations occurred below 54°F.
5. Metacercariae developed rapidly at 70°F. No development was found in fish exposed to cercariae at 70°F and held at 40-42°F for one month; however, some metacercariae developed normally when these fish were returned to 70°F.
6. Water temperature apparently plays a key role in the distribution of B. confusus metacercariae in this area.

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